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CONTEMPORARY STATE OF EXPERIMENTAL INVESTIGATIONS ON THE SPECIFIC PROPHYLAXIS OF COCCIDIOIDOMYCOSIS
(Review)

[Following is the translation of an article by S. L. Borodko, Volgograd Branch of the Rostov/Don Scientific Research Antiplague Institute, published in the Russian-language periodical Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii (Journal of Microbiology, Epidemiology and Immunobiology) No 1, 1968, pages 98-100. It was submitted on 30 May 1967.]

Over the past year interest has grown in one of the visceral mycoses - coccidioidomycosis - in connection with its detection in different latitudes of the world, apart from the USA where it is endimic (Aravivskiy and Kashkin, 1960; Shostak, 1957; Soshchek and associates, 1959; Zakharov, 1962; Stepanishcheva, 1966).

A great deal of attention is being given to the study of the morphology, biology of C. immitis, causes of the disease, clinical manifestations, means and methods of treatment, mechanisms of development of nonsusceptibility to repeated infection, and the possibility of creating postvaccinal immunity.

It has been proven epidemiologically that having an infection caused by the fungus C. immitis protects man from repeated exogenous infection. Smith (1940, 1957, 1962) and Levine and associates (1960) noted that only in an insignificant number of persons, who had acquired a cutaneous sensitivity to coccidioidin in regions of the USA which are endemic for coccidioidomycosis, did a clinically expressed disease develop.

During experimental infection of laboratory animals with the fungus C. immitis the animals acquired a resistence to exogenous reinfection but preserved the fungus in the organism for a long time (Wilmford and Gilchrist, 1896; Negroni and Bonfiglioli, 1940; Smith, 1951; citation by Levine, 1962). All of this served as the basis for searching for vaccines for the specific prophylaxis of deep mycoses. These searches are continuing up till the present time.

Testing of the effectiveness of killed vaccines against coccidioidomycosis was carried out on various laboratory animals by Negroni (1940), Vogel and associates (1954), Friedman and associates (1956), Levine and associates (1960-1962, 1965), Converse and associates (1962, 1965), and Kong and associates (1963).

Friedman(1954) and Levine and associates (1960-1962, 1965) studied the immunogenicity of formalinized vaccines from arthrogenous spores, mycelium, spherules, and a mixture of spherules with endospores of C. immitis. The vaccines were administered in various doses and methods 1-4 times with weekly intervals. In 14 days (in some cases 3-5 weeks) after the last administration of the vaccine the animals were infected with lethal doses of arthrogenous spores of the fungus C. immitis. The authors established the optimum dose of antigen (2-6 mg dry weight) which in white mice caused a high degree of resistance to intraperitoneal infection.

Immunization of mice with formalin-killed vaccine from arthrogenous spores and mycelium protected 50-70% of the animals from death during the subsequent subcutaneous or intraperitoneal infection with lethal doses of arthrogenous spores. But in 30-50% of the surviving mice upon autopsy small focal changes were revealed in the organs of the peritoneal cavity (liver, spleen), from which the causative agent was sown out. In 10-20% of the cases the fungus was isolated from those organs even in the absence of apparent pathomorphological changes in them. Thus during subsequent contamination the vaccination averted a generalization of infection from the organs of the peritoneal cavity, in spite of the fact that the causative agent was preserved in them for a long time, as this was pointed out by Converse and associates (1962).

Somewhat different results were obtained in vaccinated animals during their intranasal infection or infection by means of inhalation. Papagianis and associates (1960; citation by Levine and associates, 1960) noted that animals which had endured intraperitoneal infection died even from small doses which were administered intranasally. The organs of such animals were damaged more strongly than in immunized mice after intraperitoneal infection. Besides this, in the first case the animals sometimes displayed a generalization of the pathological process.

On the basis of the data from Aleksandrov and Gofan (1962) and others concerning the advantage of the aerosol method of vaccination over others, Sinsky and associates (1963) immunized monkeys with an aerosol of arthrogenous spores. The authors noted a lowering of the death rate in the group of vaccinated monkeys, but at the same time the development of pulmonary coccidioidomycosis in them. Castleberry and associates (1965), following the respiratory infection, with the fungus C. immitis, of dogs which had been preliminarily immunized by the aerosol method with 0.5% formaldehyde-killed arthrogenous spores, did not expose a resistance to infection in the animals; the infectious process in the vaccinated dogs was no different from that in the control. At the same time during the subcutaneous administration of the vaccine in 60% of the dogs there was increased resistance to subsequent infection by aerosols.

The authors tested vaccines which were killed by heating, carbolic tetrachloride, formaldehyde, desiccation, and other methods. The most prolonged immunity in mice was created by the vaccine from 0.5% formaldehyde-killed arthrospores.

After Converse (1965) proposed a medium for the incubation of spherules in vitro, testing began on a vaccine made from tissue elements of the fungus C. immitis on various animals.

In 3 or 5 and 8 or 12 weeks after the last immunization Vogel and associates (1954) and Levine and associates (1960-1962, 1965) infected vaccinated animals intranasally or by the aerosol route with 25-1000 LD_{50} of arthrospores. If these doses turned out to be lethal for 80-90% of the control animals, then 50-86% of the mice immunized with the spherule-endospore vaccine survived, but in 60-71% of the cases the causative agent was still preserved in the organism; in 47% of the animals the fungus was isolated from the organs without any apparent pathomorphological changes. In a similar manner was the change in experimental coccidioidomycosis in monkeys vaccinated with the spherule-endospore vaccine. In studying the spherule-endospore vaccine, many authors come to the conclusion that immunity following the administration of such a vaccine was higher than following the administration of vaccine made from mycelium and arthrogenous spores.

These same authors established that separate fractions of spherule-endospore vaccine possessed different immunogenic properties. More effective was the corpuscular fraction, and less effective - the fraction made from spherules crushed by ultrasonics. According to the data of Kong and associates (1963) the most immunogenic preparations were those made from the membranes of the spherules. Vaccines made from spherules preserved immunogenicity up to 20 months and created a specific immunity which was not weakened up to 57 days after vaccination. However, the mechanism of the protective active of spherule vaccines is still unknown up to the present time.

Kong and associates (1964) came to the conclusion that spherule vaccines protect animals from a generalized infection after the intranasal administration of lethal doses of arthrogenous spores. Here suppression of multiplication of the fungus in the organs of immune animals was noted in comparison with the organs of control animals.

Not having obtained positive results in tests with the use of killed vaccines, investigators started the testing of vaccines made from live cellular elements of the fungus C. immitis. The basis for these investigations were the data of Rixford and Gilchrist (1896), Smith (citation by Pappagianis and associates, 1961),

and Pappagianis (1959) that the infection of laboratory animals with C. immitis increased their resistance to reinfection.

In 1961 Pappagianis and associates established that in white mice the arthrogenous spores of the virulent Silveyr strain, the weakly virulent strain No 46, and the avirulent strain No 837 caused resistance to repeated infection with lethal doses of the Silveyr strain administered intraperitoneally or intranasally. However, the degree of resistance depended on the number of arthrogenous spores of the strain used for the primary infection. For the appearance of resistance in white mice it was sufficient to introduce 10^2 arthrospores of the Silveyr strain, 10^3 arthrospores of strain No 46, and 10^4-10^6 arthrospores of strain No 837. The advantage was demonstrated of the simultaneous subcutaneous-intranasal method of vaccination over each of them separately.

Thus, in the group of animals vaccinated by the combined method, after infection with 160 LD_{50} of C. immitis 100% survived, while in the group immunized only subcutaneously the death rate from such a dose comprised 50%, and in the group immunized only intranasally - 68%.

Converse and associates (1962) proposed as a vaccine the weakly virulent M-11 strain (isolated from rodents in Arizona). Animals vaccinated with 10, 10^2, and 10^3 live arthrospores of this strain, and then infected with the virulent Cash strain (10^2 arthrospores), displayed a considerably resistance to reinfection. Here they established the importance of the selection of the optimum infecting (immunizing) dose.

Converse and associates (1963, 1964) and Converse (1965) studied the possibility of the use of a live vaccine on monkeys. For these purposes various strains of C. immitis were tested, including the Silveyr (isolated from a man with acute primary infection), M-11 (isolated from rodents), Cash (isolated from a man with disseminated non-fatal infection), D-76 (isolated from dogs), and S.-1 (subculture of a human strain, possessing a low virulence and changed morphological characteristics). It was revealed that 10 arthrospores, administered under the skin, guaranteed the protection of the stated animals from subsequent respiratory infection (this was testified to by the absence of clinical or X-ray manifestations of the disease during the entire period of observation (from 3 to 10 months)). * At the same time in control monkeys, in which infection was manifested with a various clinical picture, massive foci of damage were revealed by roentgenoscopy, and upon autopsy - numerous expressed pathological changes. Thus, live vaccines, though they caused inflammatory local reactions, at the same time prevented the spreading of secondary infection beyond the limits of the regional lymph nodes.

* In the autopsy in 80% of the healthy monkeys insignificant histological changes were detected in the lungs; a culture of fungus was not obtained from the organs.

It was ascertained that the intracutaneous administration of live vaccines caused a stronger local reaction than subcutaneous, therefore the authors resorted to the subcutaneous method, and out of the 5 investigated strains of C. immitis the Cash and SH-1 strains were used.

In tests on dogs, conducted by Castleberry and associates (1964), a live vaccine from the D-76 strain of C. immitis turned out to be very effective. But in this case immunization was accompanied by strong local reactions. In connection with this investigations were conducted which were directed at blocking the local reaction with the help of antibiotics or the preliminary injection of killed vaccine; encouraging results were obtained here (Castleberry and associates, 1964; Converse, 1965).

Thus, the data which are found in available literature testify that killed vaccines (most often by 0.5% formaldehyde) did not ensure 100% protection of animals from death; they prolonged the lives of the animals during experimental coccidioidomycosis and prevented the generalization of the infection from the site of administration, but did not promote the rapid recovery of the organism from the infection.

Live vaccines from virulent, weakly virulent, and avirulent strains of C. immitis created an intense immunity in experimental animals. Here the optimum immunizing doses changed reversely proportional to the degree of virulence of the strain being tested. However, even live vaccines did not exclude as far as possible the appearance of histomorphological or apparent focal changes of weak or moderate severity in the lungs during subsequent respiratory infection or in the organs of the peritoneal cavity - following subsequent intraperitoneal infection with lethal doses of a virulent strain of this fungus./)In such cases the causative agent was isolated from the stated organs.

And so the problem of specific prophylaxis of coccidioidomycosis has still not been solved up to the present time. Killed vaccines do not completely protect one from the disease and live vaccines, though they give a more intense immunity, cause a local inflammatory reaction involving the regional lymph nodes. Such a vaccine cannot be recommended for the prophylaxis of the disease in man.

It is apparent from what was said that it is necessary to continue searches for vaccines which possess high specific immunogenic properties but do not cause reactions to administration. Apparently these searches should be conducted along the path of testing the various antigenic fractions which are obtained chemically from all the cell elements of the fungus and also by the selection of strains with weak virulence. Such strains may be found either among those isolated from nature or from man and animals which are infected with coccidioidomycosis, or they may be obtained artificially by the application of various methods on cultures of fungus (chemical, physical, and radioactive methods).

Besides this the solution of the problem of the specific prophylaxis of coccidioidomycosis should proceed along the path of studying the mechanism of immunity, since up to the present it still has not been cleared up what is the role of humoral and cellular factors of both natural and acquired immunity and what is the value of nonspecific and other factors connected with the mechanism of defense of the organism.

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